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CHALLENGES AND OPPORTUNITIES IN CREATING ARTIFICIAL BLOOD

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CHALLENGES AND OPPORTUNITIES
IN ARTIFICIAL BLOOD

ABSTRACT

Purpose: The aim of this paper is to present the current state of research on artificial blood, its potential clinical applications, and future directions of development. The problem of blood shortages in blood banks and the growing demand for transfusions pose a significant challenge to modern medicine. Consequently, scientists worldwide are attempting to develop effective blood substitutes that could act as oxygen carriers in the body and reduce reliance on traditional donors. This paper discusses various strategies for synthesizing artificial blood, taking into account advances in biotechnology, biomedical engineering, and nanotechnology. Particular emphasis is placed on research on hemoglobin carriers and synthetic erythrocytes.

Literature Source Utilization Methods: To obtain reliable and comprehensive information regarding the development of blood substitutes, a review of the available scientific literature was conducted, including databases such as PubMed, Scopus, Web of Science, and Google Scholar. The sources analyzed included scientific articles, clinical trial reports, systematic reviews, and reports on the latest technologies in biomedical engineering. This paper discusses various types of red blood cell substitutes, focusing on hemoglobin isolated from various sources (e.g., recombinant hemoglobin, invertebrate hemoglobin), synthetic microparticles that mimic erythrocytes, and modern oxygen carriers. Particular attention was paid to the results of clinical trials, which provide information on the safety, efficacy, and potential limitations of these solutions.

Scientific innovation. This article makes a significant contribution to the development of knowledge about artificial blood, presenting a comprehensive view of its potential and the challenges associated with its use in clinical practice.
Key issues are highlighted, such as the effectiveness of blood substitutes compared to natural human blood, their bioavailability, stability, and potential side effects. Furthermore, attention is paid to the production costs and the degree of acceptance of such products by the medical community and patients. The paper also points to the need for further research and the development of innovative technologies that can contribute to increased availability and safety of artificial blood. Potential directions for future research are also discussed, such as the use of nanotechnology, bioengineering, and biomaterials to develop more effective and safe blood substitutes.

Conclusions. The development of artificial red blood cell substitutes could be a breakthrough in emergency medicine and in situations where traditional transfusions are impossible or difficult (e.g., in battlefield conditions, natural disasters, or in areas with limited access to blood). Despite years of research and technological advances, many blood substitutes face challenges related to limited stability, potential toxicity, and the ability to effectively transport oxygen. Existing products that have passed clinical trials demonstrate promising properties, but they are still far from fully replacing natural blood. The study results indicate that further work on improving artificial blood should focus on improving safety, extending shelf life, and optimizing production costs. The prospects in this field are promising, and future achievements could revolutionize the treatment of patients requiring blood transfusions and contribute to improved access to healthcare worldwide.

Keywords: artificial blood, blood substitutes, clinical applications, hemoglobin carriers, oxygenating agents therapeutic, perfluorocarbons (PFCs), hemoglobin-based oxygen carriers

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ABSTRACT

Purpose: The aim of this study is to present the current state of research on artificial blood, its potential clinical applications and future directions for development. The shortage of blood in blood banks and the increasing demand for transfusions is a major challenge for modern medicine. As a result, scientists worldwide are working to develop effective blood substitutes that could act as oxygen carriers in the body and reduce dependence on traditional donors. This study discusses various strategies for synthesizing artificial blood, taking into account advances in biotechnology, biomedical engineering and nanotechnology. Particular emphasis is placed on research into haemoglobin carriers and synthetic erythrocytes.

Materials and methods. To obtain reliable and comprehensive information on the development of blood substitutes, a review of the available scientific literature was conducted, including databases such as PubMed, Scopus, Web of Science and Google Scholar. Sources analyzed included scientific articles, clinical trial reports, systematic reviews and reports on the latest technologies in biomedical engineering. The study discusses different types of red blood cell substitutes, focusing on haemoglobin isolated from different sources (eg recombinant haemoglobin, invertebrate haemoglobin), synthetic microparticles that mimic erythrocytes, and modern oxygen carriers. Special attention has been given to the results of clinical trials that provide information on the safety, efficacy and potential limitations of these solutions.

Scientific novelty. This article makes a significant contribution to the knowledge of artificial blood by providing a comprehensive overview of its potential and the challenges associated with its clinical application. Key issues such as the efficacy of blood substitutes compared to natural human blood, their bioavailability, stability and potential side effects are highlighted. It also looks at production costs and the acceptance of such products by the medical community and patients. The study also highlights the need for further research and the development of innovative technologies that could improve the availability and safety of artificial blood. Potential future research directions are discussed, including the use of nanotechnology, bioengineering and biomaterials to develop more efficient and safer blood substitutes.

Conclusions. The development of artificial red blood cell substitutes could be a breakthrough in emergency medicine and in situations where traditional transfusions are impossible or difficult to perform (eg, in combat situations, natural disasters, or locations with limited blood supply). Despite years of research and technological advances, many blood substitutes face challenges related to limited stability, potential toxicity and the ability to effectively transport oxygen. Existing products that have undergone clinical trials show promising properties, but are still far from fully replacing natural blood. Research suggests that further work to improve artificial blood should focus on improving safety, extending shelf life and optimizing production costs. The outlook in this field is promising, and future advances could revolutionize the treatment of patients requiring blood transfusions and contribute to improved access to healthcare worldwide.

Key words: artificial blood, blood substitutes, clinical applications, haemoglobin carriers, oxygen therapeutic agents, perfluorocarbons, hemoglobin-based oxygen carriers

Introduction

Modern medicine faces a huge challenge: providing adequate amounts of blood for transfusion.
The blood shortage, resulting from a decreasing number of donors, an aging population, and growing clinical needs, is forcing scientists to seek alternative solutions. One of the most promising research directions is the development of artificial blood—
a substance that could replace human blood in life-saving and healing processes (Seifried and Mueller, 2011; Gasparovic Babic et al., 2024).

The idea of artificial blood is not new. Work on substitutes that could transport oxygen began as early as the 20th century, but only recent advances in bioengineering and nanotechnology have opened up new possibilities in this field. Artificial blood has the potential to not only fill blood shortages in hospitals but also become an irreplaceable resource. tool in emergency situations such as natural disasters, wars or distant space missions (Sarkar, 2008).

The purpose of this article is to review current scientific achievements and analyze the development prospects for this groundbreaking technology. The first section introduces the composition and types of artificial blood, then discusses the current state of research and the technological challenges that remain to be overcome. The final section presents the potential benefits and future directions of development that could make artificial blood a common medical solution.

The importance of blood in the body and the challenges associated with its deficiency. Blood plays a fundamental role in maintaining human life and health. One of its key functions is transporting oxygen from the lungs to all body tissues. Oxygen not only requires life but also ensures the proper functioning of physiological processes in the human body (Rhodes et al., 2024). Blood loss

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or anemia can lead to hypoxia, which negatively impacts the functioning of organs, especially the brain, heart, and tissues. In extreme cases, this results in permanent damage or even death. Therefore, ensuring an adequate supply of oxygen to tissues is essential to maintaining the body's vital functions (Macciò and Madeddu, 2012).

Every day, many patients around the world require blood transfusions for various reasons. Unfortunately, one of the significant challenges facing healthcare systems remains the limited availability of blood and its components when they are most needed.
Despite years of research and the rapid development of medicine and technology, an effective method for producing artificial blood has yet to be developed. Currently, the human body remains its only source (Niechwia-dowicz-Czapka and Klimczyk, 2011; Osaro and Charles, 2011).

However, recent years have seen a significant decline in the number of voluntary blood donors. According to the World Health Organization's recommendations, to ensure adequate blood reserves in a given country, 2–2.5% of the population should regularly donate blood (Guidelines Review Committee, 2010). Meanwhile, in Poland, this figure is only 1.9%, while in the United States it reaches as much as 7% (Makowicz et al., 2022). This state of affairs is due, among other things, to an aging population and widespread myths about blood donation, which may discourage potential donors. The most common misconceptions include fear of becoming addicted to donating blood, fear of overproduction of blood with regular donation, and the belief that it may lead to the development of hypertension (Kozÿowska and Kempa, 2011).

Modern medicine increasingly requires blood and its components, particularly in the treatment of cancer, cardiac surgery, and transplantation. However, recruiting new donors poses a significant challenge (Thomson et al., 2009; Gasparovic Babic et al., 2024).

Additionally, adverse reactions following blood transfusions are increasingly reported, and new blood-borne infectious agents are emerging (Dellinger and Anaya, 2004; Bloch et al., 2012).

All these factors constitute strong arguments, which encourage scientists to constantly searching for alternative solutions to human blood.

History, challenges, and prospects for the development of blood substitutes. Throughout the history of medicine, the need for blood substitutes has existed since patients died from serious wounds. The first records of blood transfusions date back to the ancient Incas. However, it was not until 1616 that William Harvey described blood circulation, which became the basis for further attempts to replace blood with other substances, such as beer, urine, milk, plant resins, and sheep blood. In the 18th century, experiments with blood substitutes, such as milk or saline, had limited success and were abandoned due to questionable effectiveness or side effects (Sarkar, 2008).

Research into blood alternatives began about 150 years ago. In his search for a blood substitute, T. Gaillard Thomas proposed injecting cow's milk intravenously, which he termed "milk injections."
(Thomas, 1878). He justified his hypothesis by the chemical similarities between lymph and milk, emphasizing that both are fat emulsions suspended in fluid. Thomas presented three case studies of patients in a moribund state who were given approximately 8 ounces of fresh cow's milk. One patient survived, and two died. He attributed the deaths of these patients to other complications unrelated to the milk injections, arguing that they were safe provided fresh milk was used (Chen et al., 2009).

Despite Thomas's claims, the development Blood substitutes focused on the creation of hemoglobin solutions, which were first tested clinically in the early 20th century. The oxygen-carrying characteristics of hemoglobin made it a logical choice for blood substitutes, but its use was associated with unexpected consequences (Chen et al., 2009). Amberson and colleagues (1933) conducted experiments on cats, completely replacing their blood with cell-free hemoglobin dissolved in lactated Ringer's solution. They showed that this solution could sustain life, but the benefits were short-lived, and the treatment caused serious kidney damage. Despite these observations, clinical trials were conducted using hemoglobin in Ringer's solution, which unfortunately led to significant kidney dysfunction in 5 of 14 patients. As a result, Amberson

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son and colleagues discontinued further studies, concluding that hemoglobin solutions require further development due to their renal toxicity and induction of vascular hypertension (Chen et al., 2009).

In 1883, the discovery of Ringer's solution, which could restore blood pressure, was a major step in the development of artificial blood, although it did not replace the function of red blood cells. It was not until the 20th century, thanks to Karl Landsteiner's work on the classification of blood groups, that transfusions became safer and blood donation was implemented on a large scale (Sarkar, 2008).

In the 1950s, the U.S. Navy treated 47 anemic and febrile sailors with one or more infusions of hemoglobin solutions. Seventeen sailors developed hypertension, and 12 of the 52 infusions caused symptoms of kidney problems. The remaining sailors, who had no kidney problems, experienced other adverse effects.

Renal toxicity was likely due to renal tubular obstruction.

by hemoglobin and the erythrocyte stromal membrane, impaired renal function due to heme pigment deposition, and reduced renal blood flow due to hemoglobin-induced vasoconstriction (Winslow, 1992).

Due to evidence of renal toxicity of hemoglobin solutions, interest in them waned, and it took several years before useful hemoglobin solutions free of stromal impurities were developed.

Ultracleaning techniques, which remove stroma and other cellular debris, have largely solved the problem of kidney toxicity. However, new challenges have emerged. Once removed from red blood cells, native tetrameric hemoglobin disintegrates into dimers, which are rapidly cleared by glomerular filtration, resulting in a short vascular half-life.

Additionally, free hemoglobin has limited contact with phosphates, which causes a leftward shift of the P50 curve , resulting in high affinity for oxygen and limited ability to release it (Chen et al., 2009).

To overcome these limitations, various approaches have been used for molecular stabilization and chemical modification of hemoglobin.
In the late 1960s, researchers from the United States Army developed a promising new hemoglobin solution. Bunn and Jandl (1968) used cross-linking of hemoglobin with bis(N-maleimidomethyl) ether (BME), which prolonged its intravascular retention time.

Cross-linking reduced the tendency of hemoglobin to form dimers, which limited its renal filtration and clearance (Bunn and Jandl, 1968).

Other researchers have chemically modified hemoglobin at 2,3-DPG binding sites, amino groups, or internal structures to prevent its degradation into ÿÿ dimers and restore P50 values to near-normal levels (Winslow, 2006a,b). Bonsen et al. (1975) used a different approach: polymerization of hemoglobin with glutaraldehyde, which extended its intravascular retention time. Another method involved attaching hemoglobin to larger molecules, allowing it to remain in the vasculature longer than unmodified hemoglobin. One study demonstrated that hemoglobin bound to dextran sustained life in dogs and cats in the absence of red blood cells (Tam et al., 1976, 1978).

Research into blood substitutes resumed after World War II, when human plasma began to be used in transfusions. In 1966, the concept of using perfluorocarbon compounds (PFCs) as blood substitutes emerged. Although research on these compounds showed promise, the blood donation system developed in developed countries limited interest in further research. Interest surged after the Vietnam War, when it was discovered that transfusions could transmit HIV and viral hepatitis, which intensified the search for new synthetic oxygen carriers (Sarkar, 2008).

Blood and its substitutes: key role, properties, and modern therapeutic solutions. Blood plays a fundamental role in living organisms, ensuring the transport of oxygen from the lungs to tissues and carbon dioxide from the tissues to the lungs. The main role in this process is played by red blood cells, which naturally bind oxygen and transport it to cells. This ability results from the presence of hemoglobin in erythrocytes – a tetrameric protein capable of reversibly binding oxygen and carbon dioxide. Hemoglobin consists of two ÿ polypeptide chains (1 and 2) and two ÿ chains (1 and 2), with

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each of which contains a heme group with an iron atom, enabling the binding of one oxygen molecule (Chen et al., 2009; Khan et al., 2020).

Blood, often considered the essence of life, performs many important physiological functions thanks to its plasma components and cells. It transports oxygen and nutrients to tissues and removes waste products. White blood cells (granulocytes, B and T lymphocytes) play a key role in the immune response, while platelets, coagulation factors, and fibrinolytic factors are essential for maintaining the balance between clot formation and breakdown. Blood also transports hormones and plays a crucial role in maintaining pH balance, which must be precisely regulated at the body's level.

7.40 (InformedHealth.org, 2006).

Mature human erythrocytes are characterized by a biconcave disk shape and the absence of organelles, which distinguishes them from other cells. mammalian cells. Despite lacking a complete energy and genetic system, erythrocytes live for approximately 120 days and play a vital role in transporting oxygen to tissues. Their biconcave shape, resulting from high hemoglobin concentration and the absence of a nucleus, provides greater flexibility and a larger surface area for gas exchange, enabling more efficient transport of oxygen and carbon dioxide (Mohandas and Gallagher, 2008; Zhang et al., 2024).

The erythrocyte cell membrane responds for their mechanical, transport, and antigenic properties. Hemoglobin in the membrane transports respiratory gases, and erythrocytes are formed from stem cells in the bone marrow. The spleen and liver play key roles in erythrocyte metabolism, filtering worn-out cells and recycling iron (Zhang et al., 2024).

The characteristics of red blood cells are shown in Figure 1.

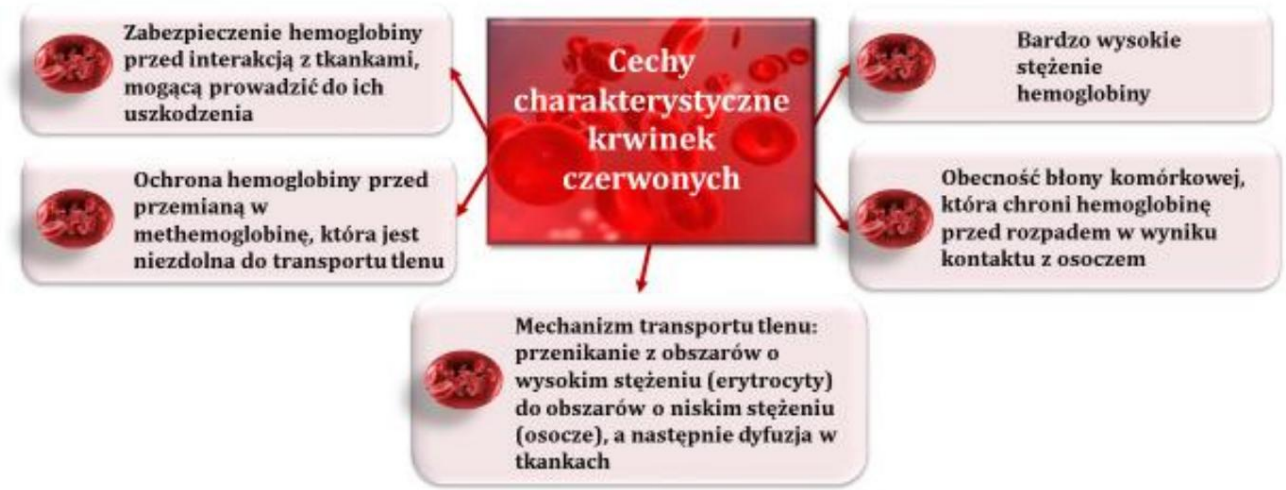
Cechy charakterystyczne krwinek czerwonych
Zabezpieczenie hemoglobiny przed interakcją z tkankami, mogącą prowadzić do ich uszkodzenia
Ochrona hemoglobiny przed przemianą w methemoglobinę, która jest niezdolna do transportu tlenu
Bardzo wysokie stężenie hemoglobiny
Obecność błony komórkowej, która chroni hemoglobinę przed rozpadem w wyniku kontaktu z osoczem
Mechanizm transportu tlenu: przenikanie z obszarów o wysokim stężeniu (erytrocyty) do obszarów o niskim stężeniu (osocze), a następnie dyfuzja w tkankach

Figure 1. Characteristic features of red blood cells (Source: Mohandas and Gallagher, 2008; Zhang et al., 2024)

Blood substitutes, despite their name, are not full-fledged blood substitutes. They are designed solely to support one specific therapeutic function: transporting oxygen to tissues.

Therefore, it is more accurate to call them “oxygen therapeutic agents” (OTAs) (Chen et al., 2009; Khan et al., 2020).

For blood substitutes to be effective and safe in clinical applications, they must meet certain criteria (Fig. 2).

Currently, research on blood substitutes focuses on three main areas (Fig. 3):

ÿSynthetic oxygen carriers such as perfluorocarbon compounds (Haldar et al., 2019; Jägers et al., 2021).

ÿHemoglobin-based oxygen carriers, produced from hemoglobin obtained from red blood cells (Jansman and Hosta-Rigau, 2018; Faggiano et al., 2022).

Artificial red blood cells that have the potential to replace natural erythrocytes in the oxygen transport process (Azuma and Sakai, 2019; Waeterschoot et al., 2024).

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Brak antygenów czerwonokrwinkowych, co umożliwia ich przetaczanie bez potrzeby wykonywania próby zgodności
Wolność od czynników zakaźnych
Dłuższy okres przechowywania w porównaniu z krwią ludzką
Substytuty krwi
Właściwości funkcjonalne zbliżone do krwi ludzkiej
Potencjalnie ulepszone właściwości reologiczne
Niski koszt produkcji i stosowania
Łatwość metabolizowania przez organizm, nawet w przypadku masywnej transfuzji

Figure 2. Criteria for the efficacy and safety of blood substitutes in clinical applications (Source: Winslow, 2002; Tappenden, 2007; Jahr et al., 2021; Chen et al., 2023)

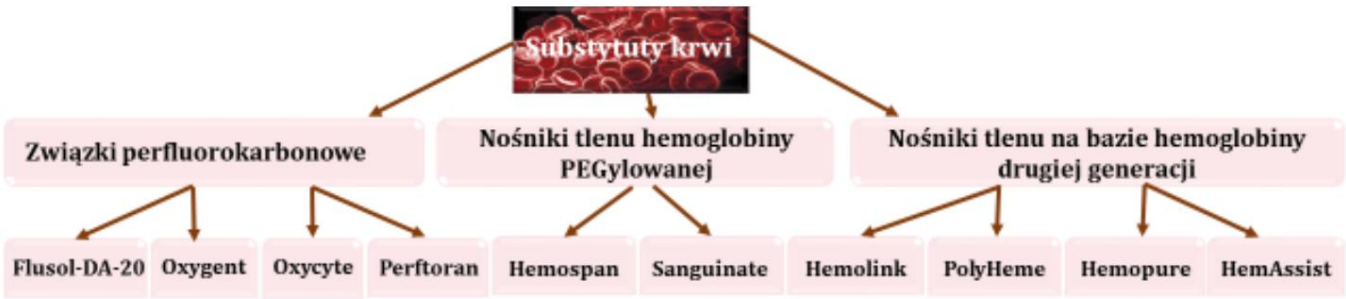

Figure 3. Main areas of research on blood substitutes (Source: Khan et al., 2020)

Types and composition of artificial blood. Artificial Blood is a promising alternative to human blood, which can be used in emergency situations such as mass casualty incidents, medical surgeries, or blood bank shortages. Currently, research on artificial blood focuses on various technological and biological approaches aimed at replicating the basic functions of blood (Sarkar, 2008; Haldar et al., 2019). The main types of artificial blood and their composition are presented below.

1. Synthetic oxygen carriers based on perfluorocarbons (PFCs)

Perfluorocarbons (PFCs) were first described as oxygen carriers by Clark and Gollan in 1966, who showed that mice could survive in a PFC solution enriched with oxygen (Clark and Gollan, 1966). PFCs are synthetic molecules composed of carbon and fluorine atoms that form strong bonds that protect against chemical degradation (Fig. 4).

Due to their hydrophobic properties, PFCs must be stabilized in emulsions for intravenous use. In this form, PFCs are excellent gas solubilizers, due to the low polarizability of fluorine and weak intermolecular forces (Lowe, 1999; Riess, 2005). They are exceptionally small—about 100 times smaller than erythrocytes—and capable of transporting oxygen and carbon dioxide without chemical binding. Their most important property is their ability to dissolve gases, which is 20-25 times greater than water or plasma, a process that occurs passively, without the involvement of chemical reactions (Lane, 1995; Cohn and Cushing, 2009).

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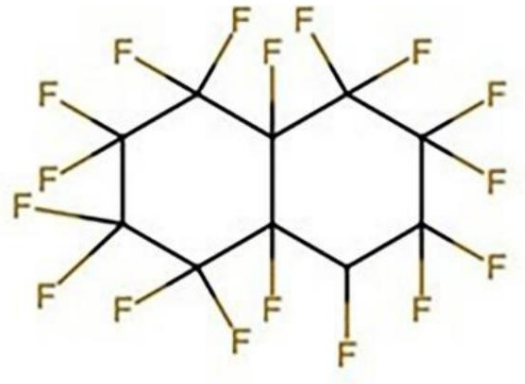

Fig. 4. Structure of a perfluorocarbon-based oxygen carrier (Source: Khan et al., 2020)

PFCs dissolve oxygen and carbon dioxide efficiently due to Henry's principle, which states that the concentration of a dissolved gas is proportional to its partial pressure.
Unlike hemoglobin, which requires chemical bonds to transport oxygen, PFCs enable the rapid and efficient release of gases when needed, regardless of temperature and environmental conditions (Biro and Blais, 1987; Shi et al., 2009).

PFCs have a linear relationship between dissolved oxygen levels and oxygen pressure, meaning that high oxygen pressure is necessary for maximum oxygen-carrying capacity. By replacing hydrogen atoms with fluorine atoms, PFCs are water-insoluble and non-metabolizable due to their strong carbon-fluorine bonds. In clinical applications, they require emulsifiers to become soluble (Riess, 2005).

PFCs are an attractive alternative for people who refuse transfusions of blood or proteins of human or animal origin.
They dissolve oxygen at a concentration of 40–50%, which is 20 times greater than in water and twice as much as in plasma. They also dissolve 130–160 ml of carbon dioxide, which is two to three times greater than water (Shi et al., 2009). Some PFCs, such as FC-80, have an oxygen-dissolving capacity greater than that of erythrocytes (by 10%).

The fundamental difference between oxygen transfer by hemoglobin (Hb) and PFCs is that Hb chemically binds oxygen, whereas PFCs dissolve it (Cohn and Cushing, 2009).
PFCs are resistant to high temperatures (up to 300°C), which allows them to be easily heat sterilized (Cabrales and Intaglietta, 2013). Due to their small size, they can pass through vessels blocked in certain diseases, where erythrocytes cannot move, which improves tissue oxygenation (Moradi et al., 2016).

In vitro studies have shown that PFCs as artificial blood are particularly useful in cases of blocked coronary arteries, helping to maintain heart muscle function (Mushlin et al., 1985). Additionally, research by Chen et al. (2013) demonstrated the effectiveness of PFCs as a blood substitute during surgery to treat traumatic and hemorrhagic shock, including in war casualties.

Molecular stability, gas-dissolving ability, and unique physicochemical properties make PFCs promising candidates for biomedical applications.

Thanks to fluorinated bonds, PFCs exhibit unique properties that distinguish them from other organic compounds and can form the basis of innovative medical solutions.
(Khan et al., 2020; Kakaei et al., 2023).

The metabolism of PFCs is mainly based on their excretion through the lungs together with the exhaled air, and partly by phagocytes of the reticuloendothelial system, especially in the spleen and liver. PFC molecules, suspended in plasma, travel between red blood cells and near blood vessel walls. In the lungs, they bind oxygen, which they then release in tissues through diffusion (Haldar et al., 2019).

PFCs are relatively cheap to produce, which can be achieved without the use of biological material, thus eliminating the risk of transmitting infectious diseases through blood transfusion. However, there are two major technological obstacles limiting their clinical use. First, they are insoluble in water, requiring the use of emulsifiers—fatty lipid compounds that allow suspension.

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fine PFC particles in the blood. Secondly, their oxygen-carrying capacity is lower than that of hemoglobin-based products, which means they must be used in larger quantities (Sarkar, 2008).

Perfluorocarbon emulsions I and II generation (Moradi et al., 2016; Khan et al., 2020):

1. Fluosol (Green Cross, Japan);
2. Perftoran (Perftoran, Russia);
3. Oxygent (China);
4. PHER-O2 (Sanguine Corp.);
5. Oxycyte (Synthetic Blood International).

Advantages and disadvantages of perfluorocarboxylic emulsions vouchers are presented in Table 1.

Table 1

Advantages and disadvantages of perfluorocarbon emulsions (Jahr et al., 2021)

Advantages of perfluorocarbon emulsions	Disadvantages of perfluorocarbon emulsions
ÿPossibility of mass production; ÿLow manufacturing cost; ÿLong storage time; ÿMinimal immunogenicity; ÿLow risk of infection.	ÿThe need to use emulsifiers, which may cause side effects; ÿRequirement to use increased oxygen concentration in inhaled air; ÿLimited oxygen carrying capacity at physiological values of oxygen partial pressure (pO2); ÿRisk of spleen and liver enlargement, flu-like symptoms and thrombocytopenia; ÿShort duration of action in the bloodstream

Fluosol-DA was the first approved PFC-based erythrocyte substitute. It was an emulsion of perfluorodecalin and perfluorotripropylamine. The oxygen carrying capacity of Fluosol-DA is only 7.2% at 37°C, which is significantly lower than that of erythrocytes (Nishimura et al., 1981). The use of this preparation has been associated with complications such as pulmonary reactions, presumably due to activation complement system by the emulsifier contained in Fluosol-DA (Bowma, 1983). These reactions could be reduced by the administration of steroids. For this reason, the efficacy of Fluosol-DA has not been confirmed in clinical trials, and its clinical use has been discontinued (Lane, 1995).

Among the PFCs, perflu-bron and perfluorodecalin have been extensively studied (Nishimura et al., 1981). Second-generation PFC-based blood substitutes include OxyFluor™ (Hemagen, Inc.) and Oxygent™ (aka perflubron, Alliance Pharmaceutical, Inc.) (Lowe, 2006). Both products were rejected in clinical trials due to side effects, such as difficulty in determining the effective dose of OxyFluor™ and an increased risk of stroke after Oxygent™ administration (Tao and Ghoroghchian, 2014). However, no significant interactions between blood components and administered PFC preparations, except for some changes in coagulation factors (Sharma et al., 2011). Administration of PFC-based products may lead to mild thrombocytopenia (a 10–15% decrease in platelet count) and symptoms similar to a flu-like syndrome (Kresie, 2001).

Due to unfavorable results in initial clinical trials of PFC-based products, no further clinical trials are currently being conducted in this area. Some products, such as Perftoran® (Russian Academy of Sciences, Pushchino, Russia), are used in Mexico and Russia. Perftoran, known in the United States as Vidophor™ (Fluor02 Therapeutics, Inc., Boca Raton, FL), is currently awaiting clinical trials (Latson, 2019).

Ultimately, due to the lack of sufficient clinical evidence and the lengthy FDA approval process, PFCs are unlikely to become a practical blood substitute in the near future (Khan et al., 2020).

2. Hemoglobin-based oxygen carriers (HBOCs) Hb-based oxygen carriers ,

Currently, intensive research is being carried out to find a synthetic substance,

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that could address the challenges of hemoglobin oxygen transport, effectively delivering Oÿ to tissues without causing toxicity. Unfortunately, due to adverse events in Phase II and III trials, none of the products developed to date have been approved for clinical use. However, hemoglobin-based oxygen carriers (HBOCs) have been approved for clinical use in Russia and South Africa (Lang et al., 1990; Stowell, 2008).

Despite these challenges, these studies have provided new insights into the fundamental biophysical characteristics of hemoglobin and oxygen transport systems, paving the way for the development of products with high oxygen carrying capacity and minimal risk of interaction with hemodynamics. In this context, hemoglobin-based products are being developed as adjuncts or pharmaceutical bridging agents to allogeneic red blood cell transfusion (Chang, 2003).

Free hemoglobin can theoretically be used as a blood substitute because it retains the ability to transport oxygen outside of red blood cells. However, its toxicity to surrounding tissues is a serious limitation. Toxicity includes nitric oxide (NO) scavenging by the endothelium, leading to vasoconstriction (vasoconstriction) and the generation of reactive oxygen species, heme, and globin. Furthermore, in the absence of 2,3-diphosphoglycerate (2,3-DPG), free hemoglobin is a very inefficient oxygen transporter, releasing only small amounts of Oÿ to tissues (Simoni et al., 2009). The first use of cell-free hemoglobin in humans was reported in 1949 by Dr. Amberson and his team, who administered a hemoglobin solution to a patient with massive postpartum hemorrhage (Amberson et al., 1949).

Due to the toxic nature of free hemoglobin and the requirement of 2,3-DPG for effective oxygen transport, the development of HBOC is a significant challenge (Fig. 5). However, the successful development of an HBOC-based blood substitute offers many advantages, such as the absence of compatibility testing prior to administration, the ability to be sterilized by ultrafiltration or pasteurization, and a long shelf life —features that are not available with traditional donor red blood cell preparations.

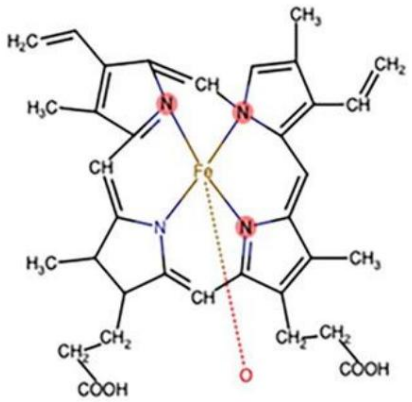

Figure 5. Structure of hemoglobin-based oxygen carrier (Source: Khan et al., 2020)

This is one of the most advanced technologies in the field of artificial blood. HBOCs are blood substitutes based on natural hemoglobin, obtained through polymerization, cross-linking, and polymer modification processes. Due to their properties similar to natural hemoglobin in terms of oxygen transport and release, they are the main subject of research among artificial oxygen carriers (Zhu et al., 2024).

The source of hemoglobin is expired, purified human or bovine blood and genetic engineering methods (Jahr et al., 2021). Oxygen binds to these compounds in the same way as it does to naturally occurring hemoglobin. Due to rapid degradation, the possibility of kidney damage, and a high affinity for oxygen, which hinders its release into tissues, hemoglobin is modified. The modification involves additional bonds within the hemoglobin molecule, between molecules

hemoglobin or with high molecular weight substances (Sarkar, 2008).

Various strategies are used to stabilize hemoglobin:

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a) Hemoglobin polymerization. This process significantly increases the size of cell-free hemoglobin, reduces extravasation, and prolongs its residence time in the circulation. Polymerization is typically performed using glutaraldehyde. Examples of polymerized hemoglobin products include HemAssist, Hemolink, PolyHeme, and Hemopure (veterinary version: Oxyglobin).

(Chen et al., 2023).

b) Cross-linking of hemoglobin. Hemoglobin can be cross-linked between the ÿ- or ÿ-chains using diaspirin or raffinose as linkers. The cross-linked tetramers stabilize the molecule and prevent dissociation of oxyhemoglobin into dimers, which are readily excreted by the kidneys. Hemoglobins that are cross-linked
inside the molecule, although they do not show a significant increase in molecular weight, they are characterized by greater stability and solubility in solutions. Theoretically, these products should have a better oxygen transport capacity than natural red blood cells (Sarkar, 2008; Chen et al., 2023).

c) Conjugation with antioxidant enzymes. Conjugation of hemoglobin with antioxidant enzymes may protect the molecule from damage induced by free radicals. Additionally, conjugation increases the size of the molecules, which reduces their removal by the kidneys and reduces their passage through vascular walls, limiting the uptake of nitric oxide.

Combining the above techniques, such as polymerization with cross-linking or conjugation, can lead to hemoglobin with improved functional and biological properties (Chen et al., 2023). Among the various Hb modifications, only nanotechnology-based polymeric hemoglobin (PolyHb) and conjugated hemoglobin have demonstrated efficacy (Chang, 2006). However, due to their short half-life in blood and side effects, most of these products have not met clinical requirements (Tao and Ghoroghchian, 2014). The main problem that limits the use of these products is their inability to convert Fe^{3}ÿ to Fe^{2}ÿ, which is an essential function of red blood cells. This results in the formation of Met-Hb, which has limited oxygen-carrying capacity.

However, it has been shown that such complications can be avoided by attaching appropriate reductants to the hemoglobin surface in these products (Tao and Ghoroghchian, 2014).

Table 2

Advantages and disadvantages of hemoglobin solutions (Jahr et al., 2021)

Advantages of hemoglobin	Disadvantages of hemoglobin solutions
solutions ÿHigh capacity to bind oxygen and carbon dioxide; ÿCan be used at physiological pO2 values ; ÿNo red blood cell antigens; ÿExtended storage time; ÿPossibility of sterilization and virus inactivation	ÿRapid elimination from the bloodstream; ÿPotential renal toxicity; ÿHypertensive effect on blood vessels; ÿPossibility of intensifying the effects of bacterial endotoxins

The Evolution of Blood Substitutes: From the First to the Third Generation. The first generation of blood substitutes was based on products containing *stroma-free hemoglobin (SFH).* These products were obtained by lysis of concentrated erythrocytes, resulting in the formation of soluble hemoglobin. This mixture was then centrifuged to remove most of the erythrocyte membrane components, resulting in SFH. This procedure allowed for the production of 500 ml of hemoglobin solution with concentration of 7 g/100 ml and physiological levels of sodium, potassium, and bicarbonate. The solution was characterized by a pH of 7.1–7.2, an osmolality methaemoglobin of 270–280 mOsm/kg, and a concentration of 7–12% of the total haemoglobin content and did not change significantly during a four-week storage period at 4°C. Production of SFH could be achieved by ultrafiltration or crystallization (Browdie and Smith, 1975).

SFH obtained by ultrafiltration contained significantly lower amounts of residual

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membrane phospholipids and a more uniform protein composition. Furthermore, these preparations were practically devoid of vasoconstrictive activity and properties that reduced myocardial contractility in *ex vivo perfusion models.* In contrast, SFH obtained by crystallization was characterized by a lower degree of purification of phospholipids and proteins, which favored the formation of protein aggregates during storage and caused side effects such as vasoconstriction and reduced contractility, which could significantly vary between production batches. Studies have shown that the ultrafiltration and size separation methods led to SFH of higher quality compared to the crystallization method (Lang et al., 1990).

The second generation of HBOCs included pyridoxylated hemoglobin and polyoxyethylene conjugates (PHPC). These products were created by chemical modification of SFH and were designed to eliminate the main disadvantages of the first generation, such as increased hemoglobin affinity for oxygen, short circulation time in the body, and nephrotoxicity (Riess, 2006). In the process SFH was pyridoxylated (vitamin B6 added) to regulate its oxygen affinity and then bound to ÿ-carboxymethyl-ÿ-carboxymethoxypolyethylene, which increased its molecular weight and prolonged its residence time in the blood (Chang, 1992).

Second-generation products included PolyHeme® (Northfield Laboratories), Hemopure® (HBOC-201, Hemoglobin Oxygen Therapeutics), and HemoLink™ (Hemosol Inc.). Although many of these products were withdrawn due to adverse events observed in clinical trials, Hemopure® received approval from the South African Drug Administration for the treatment of anemia in 2001. This product is also available in the United States under the FDA's expanded access (compassionate use) program for patients with life-threatening anemia who do not accept blood transfusions (e.g., Jehovah's Witnesses) (Jahr et al., 2007; Mer et al., 2016).

The third generation of blood substitutes included hemoglobin cross-linked between the ÿ chains using bis(dibromosalicyl) fumarates (DBBF) or ÿÿ-hemoglobin. These products were developed at *Letterman Army Institute of Research* (LAIR) in San Francisco and by *Baxter International Corporation,* which created the HemAssist® preparation based on diaspirin *-crosslinked hemoglobin,* DCLHB) (Winslow, 1995; Highsmith et al., 1997; Jahr et al., 2007; Vincent et al., 2015). Third generation products were made from expired human or bovine blood. Red blood cells were washed in sterile saline to remove residual plasma and then subjected to hyperonic lysis. Residual cell membranes were filtered to yield purified hemoglobin, which was cross-linked to achieve an oxygen-carrying capacity of 55–58% (Nelson et al., 1992; Jahr et al., 2007). The final products demonstrated oxygen-carrying properties similar to human blood; however, increased mortality was observed in Phase III clinical trials, which limited their use (Jahr et al., 2007).

Despite these limitations, Hemolink™ stands out as a modern product based on hemoglobin cross-linked with activated sugar (O-raffinose). It exhibits specific physicochemical properties, such as such as a P50 pressure of 30–40 mmHg and lack of cooperativity in oxygen binding (Hill coefficient n = 1). This product was tested in phase I and II clinical trials in patients undergoing elective coronary artery bypass graft (CABG) surgery, however, the lack of available data from phase III studies limits its further use (Carmichael et al., 2000; Hill et al., 2002; Boykins et al., 2005).

Hb-based oxygen carriers (HBOCs) are divided into two groups: acellular HBOCs and cellular HBOCs (Figures 6 and 7). Acellular HBOCs have been developed to increase the efficiency of Hb and reduce its side effects (Moradi et al., 2016). Currently, they are in various phases of clinical trials and fall into three categories, including cross-linked HBOC, polymerized HBOC, and conjugated HBOC (Figure 6). However, among the various Hb modifications, only nanotechnology-based polyhemoglobin (PolyHb) and conjugated Hb are effective (Chang, 2006). However, due to their short blood half-life and side effects, most of these products did not reach the required criteria in clinical trials (Tao and Ghoroghchian, 2014).

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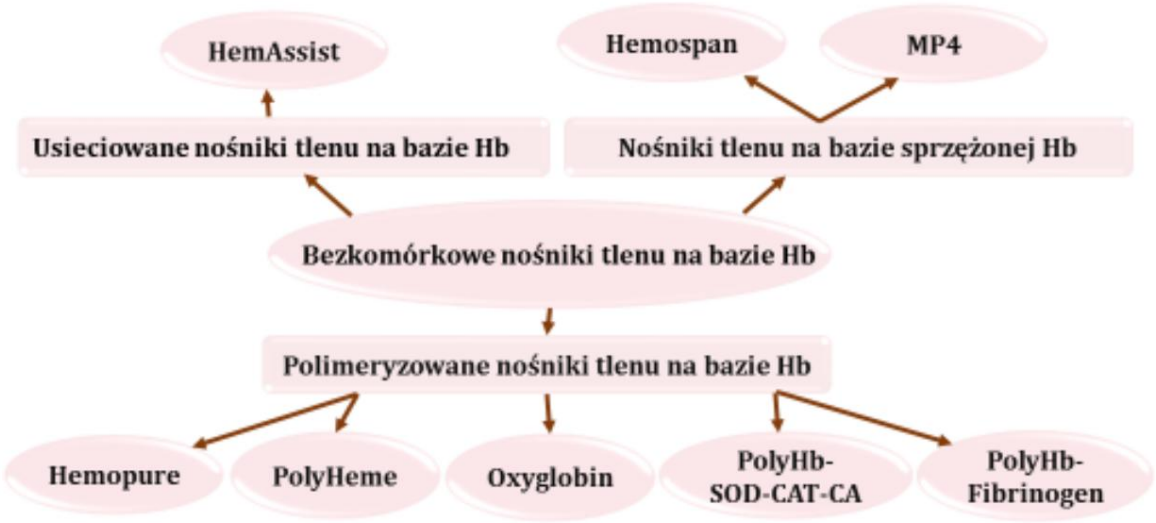

Fig. 6. Cell-free oxygen carriers based on hemoglobin (Moradi et al., 2016)

Other types of hemoglobin-based products used as artificial blood are cellular oxygen carriers, in which hemoglobin is encapsulated in a cell-like structure. This has resulted in products with the highest similarity. to red blood cells that do not cause vascular activation due to nitric oxide (NO) uptake. A summary of the products corresponding to hemoglobin-based cellular products is presented in Fig. 7.

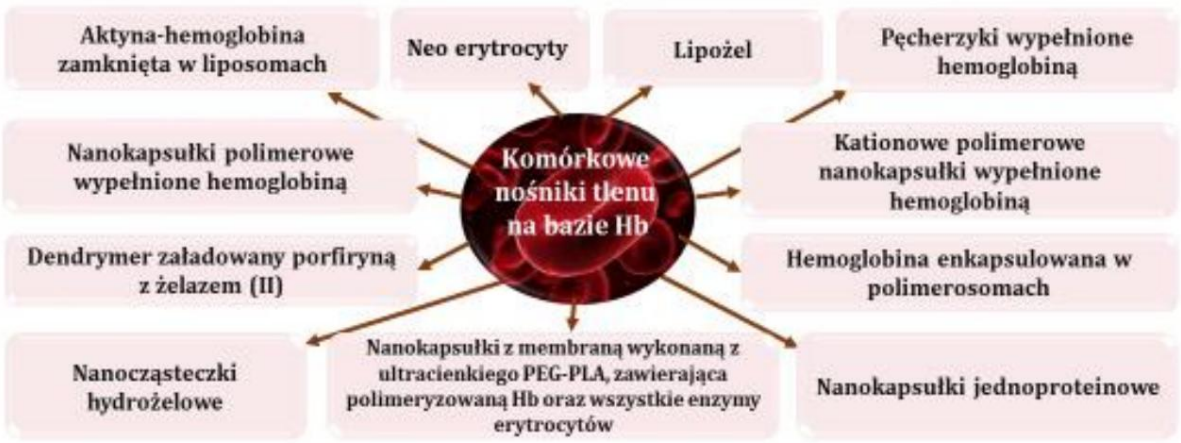
Aktyna-hemoglobina zamknięta w liposomach
Neo erytrocyty
Lipożel
Pęcherzyki wypełnione hemoglobiną
Nanokapsułki polimerowe wypełnione hemoglobiną
Komórkowe nośniki tlenu na bazie Hb
Kationowe polimerowe nanokapsułki wypełnione hemoglobiną
Dendrymer załadowany porfiryną z żelazem (II)
Hemoglobina enkapsulowana w polimerosomach
Nanocząsteczki hydrożelowe
Nanokapsułki z membraną wykonaną z ultracienkiego PEG-PLA, zawierająca polimeryzowaną Hb oraz wszystkie enzymy erytrocytów
Nanokapsułki jednoproteinowe

Fig. 7. Hemoglobin-based cellular oxygen carriers (Moradi et al., 2016)

Recombinant Hb. Hemoglobin is most active when present in red blood cells. Therefore, if Hb is to be used in a cell-free form, it must undergo numerous modifications to increase its half-life in circulation and prevent related complications in the body.

Recombinant production of hemoglobin facilitates the introduction of modifications, especially by site-directed mutagenesis (Fronticelli and Koehler, 2009).

In the 1980s, mass production of human hemoglobin in transgenic organisms began. Since then

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Research is being conducted to improve the quality and efficiency of recombinant hemoglobin production. It should be emphasized that most studies have focused on hemoglobin production using an *E. coli* expression system (Hoffman et al., 1990; Varnado et al., 2013). However, hemoglobin has also been expressed in other bacterial systems, as well as in transgenic mice and pigs (Varnado et al., 2013).

However, the most important problems in recombinant hemoglobin production are low expression yields, high costs of production processes and difficulties in achieving the expected product purity (Varnado et al., 2013).

Many attempts have been made to increase the efficiency of recombinant hemoglobin production. For example, one study used an *E. coli* expression system to produce large quantities of human ÿ-globin and bovine ÿ-globin for therapeutic purposes (Hoffman et al., 1990). The resulting hemoglobins (Hb Minotaur) were then polymerized via intermolecular disulfide bonds to form Hb Polytaur. Animal studies have shown some advantages of this product over other similar products (Bobofchak et al., 2003).

It has also been proposed that mutations in hemoglobin chains (ÿGly16 ÿ Ala and ÿGly15 ÿ Ala) may increase hemoglobin production efficiency by preventing degradation and aggregation of the expressed chains. Furthermore, to increase the efficiency of heme production, necessary for the production of functional hemoglobin, a strategy of co-expressing a heme transporter in the bacterial membrane could be employed. This approach may increase heme uptake by the bacterial host and thus improve hemoglobin production efficiency (Graves et al., 2008).

Targeted mutations of hemoglobin can also improve its function outside of erythrocytes. For example, they can increase its affinity for oxygen, reduce its ability to bind NO, limit autoxidation, reduce the rate of heme loss, prevent subunit dissociation, and limit irreversible subunit denaturation (Varnado et al., 2013).

In another study, rHb (ÿN108Q) was produced using an *E. coli expression system.* In this version of hemoglobin, the N ÿ Q mutation occurred at position 108 of the ÿ chain, which is located at the interface of the ÿ1ÿ1 subunits and in the central cavity of hemoglobin. This mutation results in decreased oxygen affinity, increased cooperativity, and decreased autoxidation of the product (Hoffman et al., 1990; Tsai et al., 2000).

These are just some of the examples of successful attempts to produce recombinant HBOCs that could be used as potential blood substitutes. However, none of these products have been licensed for therapeutic use (Varnado et al., 2013).

Examples of preparations containing modified hemoglobin (Moradi et al., 2016; Khan et al., 2020):

ÿPHP (Apex Bioscience) – human;
ÿHemAssist (Baxter) – human;
ÿHemopure (Biopure) – animal (bovine);

ÿPEG-hemoglobin (Enzon) – animal (bovine);
ÿHemolink (Hemosol) – human;
ÿPolyHeme (OPK Biotech) – human;
ÿOptro-rHb-1 (Baxter) – recombinant;

ÿNo red cells (Terumo) – human;
ÿHemospan (Sangart) – human.

3. Artificial red blood cells

Hemoglobin-based oxygen carriers are currently the subject of intensive research due to their unique properties, which are similar to the functions of natural red blood cells.

from humans or animals can be encapsulated in bilayer phospholipid capsules that mimic the erythrocyte cell membrane. Adding cholesterol molecules to the phospholipid structure provides greater stiffness and mechanical stability of the hemoglobin placed in liposomes (Moradi et al., 2016).

This process involves encapsulating hemoglobin molecules in liposomes, also known as hemoglobin vesicles (HbV). Lipid or biodegradable polymer microcapsules, which can be obtained through organic conjugation of nanomaterials with hemoglobin, replicate the structure of natural erythrocytes while providing additional benefits, such as reducing vascular activity and improving biosafety (Sakai et al., 2009).

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Encapsulation of hemoglobin in a phospholipid layer (liposome-encapsulated hemoglobin (LEH)) has been shown to extend the half-life and shelf life compared to cell-free products. LEH particles are much smaller than erythrocytes (1:30), which allows them to penetrate areas of the body that erythrocytes cannot reach. Consequently, they can pass through clots and blockages, leading to improved oxygenation in stroke (Sharma et al., 2011). However, this product has a short half-life in circulation, which can be improved, for example, by PEGylation of the particle surface.

(Sharma et al., 2011).

One study developed liposome-encapsulated hemoglobins, termed "neo-erythrocytes," whose efficacy as artificial erythrocytes was confirmed in animal total extracorporeal circulation studies. The results indicated that these products could deliver even more oxygen than native erythrocytes (Usuba et al., 1995).

Surface modification of these liposomes, including PEGylation, leads to products with extended half-life, greater stability and solubility, as well as reduced antigenicity and immunogenicity. "Hb vesicle" is a PEGylated product characterized by a long serum half-life and reduced recognition by the immune system (Sakai et al., 2001; Sou et al., 2005).

The short-lived serum LEH is due not only to its elimination by the reticuloendothelial system but also to liposome destruction caused by shear forces in the bloodstream. To address this issue, an actin matrix was incorporated into the aqueous core of submicron liposomes, increasing their mechanical strength. This strategy resulted in a prolonged half-life of the product, known as LEAcHb (Li et al., 2005).

Another group of products that act as erythrocyte mimics are biodegradable polymer nanoparticles loaded with hemoglobin (HbPNP). The biggest problem associated with their use is rapid removal by phagocytes, either directly or via opsonins. To address this problem, a number of studies have been conducted, including an experiment that showed that changing the surface charge cationized cetyltrimethylammonium bromide increases the half-life of PEGylated HbPNPs. It has been observed that the surface charge has a significant effect on the circulation time of these products – anionized HbPNPs are rapidly cleared from the bloodstream, whereas cationized HbPNPs have a long half-life (Xu et al., 2009).

Another solution that offers biocompatibility are dendrimers – products with a branched molecular structure. Synthetic dendrimers include polypropylene, poly(amino ammonium chloride), and polyester (Scott et al., 2005). Some studies have prepared dendrimers with porphyrin and Fe(II) to mimic the function of hemoglobin. The shape and size of these products resemble hemoglobin, and their ability to bind and release oxygen has been demonstrated. However, dendrimers are time-consuming and expensive to produce. Therefore, a new type of dendrimers, so-called hyperbranched polymers, has been developed, which, after appropriate modifications, can be used as oxygen carriers, while reducing the production difficulties (Twyman and Ge, 2006; Twyman et al., 2012). Dendrimers are also used in drug encapsulation, suggesting their potential use as artificial oxygen carriers through hemoglobin encapsulation (Astruc et al., 2010).

Nanocapsules with a membrane composed of ultrathin polyethylene glycol–polylactic acid (PEG–PLA), containing polymerized hemoglobin and all erythrocyte enzymes, have also been studied as a hemoglobin-based erythrocyte substitute (Chang et al., 2003). Compared to a previous product using only PLA membranes, this method demonstrated a significant increase in the product's half-life due to reduced phagocytosis, as confirmed in mouse studies (Chang et al., 2003; Sheng et al., 2009). The high circulation time and the presence of enzymatic systems, including reductase, are the most important advantages of this system. The presence of the enzymatic system, especially reductase, prevents the accumulation of methemoglobin (Chang et al., 2003).

A new class of materials for hemoglobin encapsulation includes lipogels and NHPs (hydrogel nanoparticles). NHPs use non-biodegradable but biocompatible

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Flexible hydrophilic polymers that encapsulate hemoglobin in robust, spherical hydrogel nanoparticles. Numerous complications associated with hemoglobin release following degradation of biodegradable polymers have fueled the growing interest in non-biodegradable polymers. Lipogels are NHPs encapsulated in a lipid bilayer, representing a combination of a hydrogel and a liposome. They are created by photopolymerization of poly(N-isopropylacrylamide) and polyacrylamide monomers. This approach enhances the mechanical strength of the liposomal system. In essence, this product combines the advantages of a hydrogel and a liposome, characterized by high hemoglobin loading capacity and low susceptibility to recognition by the reticuloendothelial system (Patton and Palmer, 2005).

Enhanced hemoglobin loading capacity, even higher than that of lipogels and NHPs, has been demonstrated in polymersomes encapsulating hemoglobin (PEH). This product consists of biodegradable, biocompatible amphiphilic diblock copolymers and is used to encapsulate human or animal hemoglobin. PEHs can be easily produced in large quantities, and their affinity for oxygen is comparable to that of human erythrocytes.

The mechanical strength and the ability to change size and thickness are higher in PEH than in liposomes, and these products can withstand temperatures up to 60°C.

They are stable in saline for several months and in blood for at least five days. Accumulating evidence suggests that these products may herald the emergence of an ideal artificial oxygen carrier (Arifin and Palmer, 2005; Rameez et al., 2008). In single-protein nanocapsules, the protein molecule is encapsulated in a thin polymer layer, and the resulting product exhibits mechanical, thermal, and pH stability. This method has also been applied to hemoglobin (Hegedüs et al., 2014).

Another oxygen carrier was developed by a research group in which hemoglobin was conjugated with biodegradable polymeric micelles (Shi et al., 2009). The micelle is a triblock copolymer

PEG–PMPC–PLA, in which PEG acts as the outer shell, PMPC (PLA-b-poly(2-methacryloyloxyethylphosphorylcholine)) contains propargyl groups as the middle layer, and PLA is the core layer. After these micelles are produced in aqueous solution, Hb groups bind to the micelles via propargyl groups, which makes the Hb surrounded and protected by PEG chains (Shi et al., 2009).

The dynamic development of nanotechnology and nanomaterials has created new opportunities in research on hemoglobin-based oxygen carriers. Nano-HBOCs, hemoglobin carriers developed using nanotechnology, are characterized by improved stability.

structural properties, reduced vasoactivity, extended half-life, and improved biocompatibility. However, despite significant progress, Nano-HBOCs face significant limitations: they require complex production processes and high financial costs. Therefore, developing simpler, safer, and more cost-effective methods for producing Nano-HBOCs remains a major research direction (Zhu et al., 2024).

Replicating the functions and properties of natural red blood cells in a Nano-HBOC design poses a significant technological challenge. Currently, most of these solutions are still in the laboratory stage, not yet reaching clinical trials. A comprehensive assessment of Nano-HBOC's potential as a red blood cell substitute

is therefore essential. The development of this type of technology could have a huge impact on medical progress, offering a revolutionary approach to treating patients requiring blood transfusions, thus significantly improving the quality of life (Zhu et al., 2024).

The introduction of hemoglobin-containing nanoparticles has contributed to the elimination of many negative side effects, such as toxicity associated with the presence of free hemoglobin, and has also increased oxygen-carrying capacity and efficiency due to the possibility of placing more hemoglobin in capsules (Jahr et al., 2021).

The advantages and disadvantages of artificial red blood cells are presented in Table 3.

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Table 3

Advantages and disadvantages of artificial red blood cells (Jahr et al., 2021)

Advantages of artificial red blood cells	Disadvantages of artificial red blood cells
ÿLonger time in circulation compared to perfluorocarbon preparations and some hemoglobin solutions; ÿNo toxicity related to the presence of free hemoglobin in plasma; ÿPossibility of modifying the function of hemoglobin in microcapsules by adding selected erythrocyte enzymes; ÿPossibility of simultaneous transport of medicinal substances in microcapsules with hemoglobin	ÿInsufficient data on complications such as complement activation or microcapsule elimination by the reticuloendothelial system; ÿFailure to enter clinical trials; ÿHigh technological complexity, which extends the time needed to develop final products

Progress and challenges in the development of artificial blood. For over a century, intensive research has been underway to develop artificial blood that could potentially benefit clinical medicine. Despite significant advances in medical science and technology, a universally accepted human blood substitute has yet to be developed (Khan et al., 2020). Some of the products currently undergoing clinical trials include:

1. ***HemAssist.*** This is the first modern oxygen carrier developed by Baxter (USA) in collaboration with the US Army. The product was a tetrameric hemoglobin in which two ÿ subunits (ÿ-ÿ) were cross-linked with 2,3-diaspirin, obtained from expired human blood (Sloan et al., 1999). HemAssist was well tolerated by patients in Phase I and II clinical trials. Furthermore, in Phase III trials, it demonstrated blood-sparing effects.

However, surgical patients participating in this phase were observed to have an increased risk of pancreatitis and myocardial infarction. Phase III trauma trials conducted in the United States demonstrated higher mortality in the treatment group compared to the control group, although in the European trials of the same phase, mortality was more evenly distributed between groups. The HemAssist research program was terminated in 1999 (Sloan et al., 1999; Chen et al., 2023).

2. ***PolyHem.*** PolyHeme is a product developed by Northfield Laboratories (Evanston, IL, USA) after the end of the war in PolyHeme was developed in Vietnam, using expired human blood as a raw material. PolyHeme completed all stages of clinical trials – Phases I, II, and III. The product effectively raised plasma hemoglobin levels in patients who received PolyHeme instead of allogeneic human blood transfusions. However, in Phase III clinical trials, PolyHeme failed to meet the criteria for 30-day mortality. In May 2009, the FDA rejected the product's biologics license application. As a result, Northfield Laboratories filed for bankruptcy, ending the product's path to clinical use (Chen et al., 2023).

3. ***HemoLink.*** HemoLink was developed by Hemosol Inc. (Mississauga, Canada) as a unique hemoglobin solution containing stabilized tetrameric hemoglobin A units that were cross-linked using polyaldehyde polymers of o-raffinose (Prempeh and Cheng, 2022). The product showed promising results in Phase III clinical trials, saving the lives of some patients. However, HemoLink was not approved for marketing in Canada. In 2003, Hemosol halted clinical trials and filed for bankruptcy in 2005 (Jahr et al., 2021).

4. ***Hemopure (Hb-201, Oxyglobin).*** Hemopure was developed by Biopure Corporation (Cambridge, MA, USA) as a chemically stabilized, cross-linked bovine hemoglobin dissolved in saline solution, intended for use in humans. Biopure also developed an analogous product called Oxyglobin, approved for veterinary use in dogs in the USA in 1997 and in Europe in 1998. Hemopure obtained

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approval in South Africa in 2001 and in Russia in 2006 (Waters et al., 2022). Additionally, the product has received FDA approval for expanded use in the US.

Hemopure has been evaluated in clinical trials in cardiology, where it has been shown to increase coronary oxygenation and perfusion. The product stands out for its ability to deliver oxygen to ischemic tissues without the need for pulsatile blood flow, thanks to the small size of the polymer and its unique ability to bind oxygen in the lungs and release it at the tissue level. Hemopure appeared to be safe.

in patients under 80 years of age who did not suffer from severe comorbidities.

Unfortunately, Biopure Corporation does not received full FDA approval for clinical use of the product, which led to the declaration of bankruptcy protection in 2009. That same year, the company sold its assets to OPK BioTech (Cambridge, MA, USA), and in 2014, the product was acquired by Hemoglobin-O2 Therapeutics (Souderton, PA, USA). The new owner continued to develop Hemopure.

A recent study sponsored by Hemoglobin-O2 Therapeutics showed that Hemopure could potentially restore cerebral circulation and cellular function up to four hours after death in animal models. The results, published in the April 2019 issue of the journal Nature, showed a significant reduction in cell death and restoration of some brain cell functions, including spontaneous synaptic activity.

Hemopure was a key component in the process of delivering oxygen to the brain. Further studies on the dynamics of cell regeneration following prolonged global ischemia confirmed preliminary preclinical and clinical data demonstrating Hemopure's ability to perfuse organs such as the kidneys and liver.
This suggests that Hemopure has the potential to replace red blood cell concentrates in organ transplantation (Waters et al., 2022; Chen et al., 2023).

5. ***HemO2life.*** HemO2life was developed by Hemarina in France as a product based on natural extracellular hemoglobin isolated from the marine worm *Arenicola marina.* The unique properties of this product make it a potential new therapeutic agent, providing tissue oxygenation in various situations.

(Zal et al., 2022). HemO2life is also used to preserve donor organs prior to transplantation, supporting their function in preclinical and clinical settings, and has been approved in the European Union. The product has been shown to be safe as an additive to organ preservation solutions and also has a beneficial effect on ischemia-reperfusion injury. It has numerous potential advantages in clinical practice and other applications (Zal et al., 2022).

The marine annelid *Arenicola marina* is a very ancient species that has existed on Earth for 450 million years and colonizes the intercoastal areas of the eastern Atlantic coast, from the North Sea to Biarritz. HemO2life has several unique properties:

ÿnatural extracellular hemoglobin, polymerized, with a molecular weight approximately 50 times greater than human hemoglobin,

ÿfunctional oxygen binding and releasing properties similar to human hemoglobin,

ÿability to bind 156 oxygen molecules compared to 4 in the case of human hemoglobin,

ÿnatural antioxidant properties due to innate superoxide dismutase-like activity.

In the future, it is hoped that HemO2life will gain approval for the treatment of anemia in the European Union and other countries. This product has also been tested in patients with COVID-19 and has been shown to increase arterial oxygen content when gas exchange in the lungs was insufficient, as oxygen binding and release occurred passively in a simple oxygen gradient. HemO2life may have improved survival rates for COVID-19 patients by minimizing the need for endotracheal intubation, shortening the duration of oxygen supplementation, and enabling the treatment of many patients in ventilator-deprived settings (Lupon et al., 2021).

6. ***Hemoglobin vesicles (HbV).***
Hemoglobin vesicles *(HbV)* are structural carriers of hemoglobin with a cellular configuration, containing a purified and concentrated hemoglobin solution enclosed in pegylated phospholipid vesicles. HbV have a liposomal configuration, and their particle diameters range from 225 to 285 nm, which allows for neutralization of potentially toxic effects.

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hemoglobin molecules thanks to an artificial lipid bilayer that imitates the erythrocyte cell membrane (Sakai et al., 2022).

Hemoglobin vesicles have been extensively described as a resuscitation fluid in animal models, as well as in studies on pharmacokinetics, biodistribution, excretion, immune response and hematology.

A first-in-human phase I clinical trial, conducted in 2020–2021, assessed the safety and pharmacokinetics of HbV. The results demonstrated that this product is safe and effective. HbV may potentially be used as an alternative to allogeneic blood transfusion, as well as in oxygen and carbon monoxide therapy, as a perfusate for organ transplantation, and as a photosensitizer (Frost et al., 2018; Sakai et al., 2022). Dr. Sakai's group in Japan has also received approval to conduct a phase I clinical trial (Sakai et al., 2022).

7. ***Optro.*** Optro was developed by Somatogen Inc. in San Diego, California, USA, and Eli Lilly in Indianapolis, Indiana, USA. It is a genetically modified, recombinant hemoglobin cross-linked with glycine. Optro is a product obtained through recombinant DNA technology, in which modified human hemoglobin genes were expressed in cells such as *Escherichia coli* and yeast (Jahr et al., 2021; Burhop, 2022).

Certain amino acid sequences of natural human hemoglobin genes were modified to minimize dissociation of hemoglobin molecules into dimers in the bloodstream and to preserve their affinity for oxygen when using recombinant DNA technology. The hemoglobin genes were introduced into *E. coli* cells using a plasmid vector, and expression of these genes induced production of hemoglobin protein.

inside *E. coli cells.* This technique is not cheap, and the high production cost is one of the main challenges for mass production of hemoglobin. Optro was the first product in this category (Burhop, 2022).

Future studies will be directed at selecting different combinations of multiple mutations that would limit nitric oxide scavenging, oxidative degradation, and denaturation without negatively impacting oxygen delivery (Burhop, 2022). The half-life of Optro ranged from 2 to 19 hours. Although the product was relatively well-tolerated by patients, it did not meet FDA criteria and was withdrawn in 1999 due to the capture of oxide nitrogen.

8. ***Hemospan.*** Hemospan, developed by Sangart Inc. in San Diego, California, USA, is human hemoglobin conjugated with polyethylene glycol (PEG), characterized by increased molecular size and improved oxygen affinity properties. Initial clinical trials have yielded promising results, and Hemospan did not cause severe vasoconstriction like other HBOC products. This is due to the conjugation of hemoglobin to a soluble PEG polymer.

Due to the high hydration level of PEG solutions, hemoglobin molecules have a larger molecular radius, which prevents them from passing through intercellular junctions. Therefore, Hemospan has a lesser effect on nitric oxide uptake and causes less vascular constriction and hypertension.

Despite promising results, Hemospan failed to meet clinical requirements in Phase III trials as a blood substitute and was withdrawn in 2015 (Smani, 2008). In Phase I and II trials, it was well tolerated by volunteers and patients, without causing serious adverse events such as hypertension or gastrointestinal upset. Two Phase III trials, conducted in Europe and the USA, compared Hemospan with Voluven for its effectiveness in intraoperative volume expansion and hypotension prevention. The results of these studies did not meet expectations, and it is believed that the clinical trial design was inadequate. Hemospan was not developed primarily as a volume expander, but as a therapeutic oxygen agent (Smani, 2008).

9. ***Sanguitate.*** Sanguinate, developed by Prolong Inc. in South Plainfield, New Jersey, USA, is a unique form of PEG-modified bovine hemoglobin. It represents the latest generation of hemoglobin-based oxygen carriers. PEGylation of hemoglobin under aerobic conditions significantly alters its structural and functional properties, activating tetramer dissociation, increasing oxygen affinity, and eliminating oxygen binding cooperativity (Romito et al., 2022).

PEGylation under anaerobic conditions produces an HBOC with low oxygen affinity that retains oxygen binding cooperativity, does not cause hypertension in an animal model, and remains 99% in plasma for 6 hours.

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In situations where very low oxygen tensions occur in tissues, high-affinity HBOC can optimize oxygen delivery (Romito et al., 2022).

Sanguinate's structural features and altered hemoglobin affinity for oxygen allow it to bypass microcirculation barriers, effectively delivering oxygen to ischemic tissues (Cooper et al., 2021). Sanguinate also has the ability to endogenously deliver carbon monoxide, which reduces inflammation, mitigates ischemia reperfusion injury, and activates vasodilation.

Although only Phase I trial results have been published, several Phase II trials have also been completed, suggesting a possible risk of myocardial damage, though not conclusively indicating that Sanguinate was responsible. Larger studies are needed to further investigate this causal relationship. Sanguinate has been used in emergency situations in over 100 patients with severe anemia where blood transfusion was contraindicated. Additional therapeutic effects, such as anti-inflammatory, antispasmodic, and plasma volume-expanding effects, may make Sanguinate useful in treating disorders where blood is ineffective, such as stroke, inflammatory diseases, or sepsis. For this reason, Sanguinate is more commonly referred to as a resuscitation fluid (Cooper et al., 2021).

10. ***HemoxiMer.*** HemoxiMer, developed by Apex Bioscience in North Carolina (USA), is a chemically modified human hemoglobin, a conjugate of pyrodixalated hemoglobin and ethylene polyolefin (PHP). For this reason, HemoxiMer is sometimes referred to as PHP/ HemoxiMer. Initially developed as a preparation for hemoglobin-based oxygen therapy, it later focused on its use as a nitric oxide scavenger in pathological conditions characterized by high levels of this gas (Priavalle and De Angelo, 2022).

HemoxiMer has undergone Phase I and II clinical trials, and two Phase III trials (one in the US, the other in the EU) have been partially completed. Unfortunately, due to Phase III failure, development of HemoxiMer was halted. However, clinical trials have demonstrated that HemoxiMer exhibits activity as a nitric oxide scavenger (Wollocko et al., 2022).

11. ***OxyVita.*** OxyVita is a next-generation polymeric HBOC derived from bovine hemoglobin. It was originally invented at the University of Maryland in 1999 and subsequently developed by OXYVITA Inc. in Middletown, NY.

OxyVita is manufactured using patented *zero-link polymerization technology,* which uses activators instead of binding agents to create a superpolymer composed of hemoglobin tetramers. OxyVita is a superpolymer with a molecular weight of approximately 17 MDa.

The goal of OxyVita was to improve oxygen delivery in situations where red blood cell transfusion was needed but unavailable or undesirable. The product was designed to address the challenges associated with previously ineffective HBOCs (Wollocko et al., 2022).

OxyVita has been tested by many independent research teams at various institutions over the last two decades (Abutarboush et al., 2014), and some research projects are still actively ongoing.

Despite numerous trials and partial successes, full approval of products as human blood substitutes remains a challenge. These technologies face challenges related to technological, regulatory, and financial constraints. Further research is necessary to develop effective, safe, and accessible solutions that address growing clinical needs.

Selected Aspects of Artificial Blood Research: Limitations, Progress, and Development Prospects. Despite advanced research into artificial blood, this technology is not yet capable of fully replacing all the functions of human blood, particularly in terms of efficient oxygen transport to tissues, which is a fundamental function of blood. Although the results of research to date are promising, a completely safe substitute has yet to be developed. Blood products often lead to serious health complications.

Side effects and limitations of blood substitutes:

ÿPerfluorocarbon compounds – Metabolites of these substances can remain in the body for many months, which is associated with the risk of low platelet count, fever and

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increase the risk of stroke. They are also characterized by a short storage life and limited efficiency in oxygen transport (Pidcoke et al., 2022)

ÿHemoglobin-based substitutes – Hemoglobin-based preparations cause vasoconstriction, leading to tissue hypoxia and systemic and pulmonary hypertension. Furthermore, these substitutes intensify the production of free oxygen radicals, contributing to oxidative stress. Other side effects include nephrotoxicity, gastrointestinal disturbances, and flu-like symptoms (Yu et al., 2009; Khan et al., 2020).

The limitations of blood substitutes also include their short duration of action – most hemoglobin-based preparations remain in the body for only 20–30 hours,
whereas whole blood can function for approximately 34 days. Additionally, these substitutes cannot replicate blood's ability to fight infection and form clots, limiting their use to short-term interventions (Baron, 1999).

Progress in artificial blood research.
Despite the numerous challenges associated with developing a fully-fledged human blood substitute, research continues, and new technological and biotechnological solutions demonstrate promising properties.

1. ***Erythromer –*** Innovative biosynthetic hemoglobin in the form of lipid-peptide nanocapsules that encapsulate human hemoglobin. This product is characterized by a long shelf life and the possibility of rapid reconstitution, making it particularly useful in combat conditions. Erythromer is currently in preclinical development, and clinical trials are planned for the end of 2024 (ErythroMer, 2024).

2. ***NanoSanguis –*** A Polish company is developing synthetic microparticles imitating erythrocytes, which can act as a temporary substitute for human blood in the event of its significant loss (https://nawa.gov.pl; https://ec.europa.eu/).

3. ***Alternative sources of hemoglobin –*** Hemoglobin obtained from invertebrate organisms such as earthworms *(Lumbricus terrestris)* and marine polychaetes *(Arenicola marina) is of interest.*

The hemoglobin of these organisms lacks a cell membrane, making it a potential carrier of oxygen in human blood.

preliminary studies have shown its safety in laboratory animals (Batool et al., 2021).

Arenicola marina hemoglobin (M101), commercially known as HEMO2life® produced by Hemarina (Morlaix, France), effectively delivers oxygen *in vivo* (Le Gall et al., 2014; Moon-Massat et al., 2017). Studies have shown that it does not cause oxidation, vasoconstriction, or hypertension (Rousselot et al., 2006; Tsai et al., 2012). Due to the large heme pockets in the *A. marina hemoglobin molecule,* oxygen is readily and passively released (Gow et al., 2005; Xu et al., 2006).

It should be noted that *A. marina* hemoglobin has a significantly higher affinity for oxygen than human hemoglobin, which is due to its lower p50 value of 7.05 ± 0.93 mmHg.
As a result, its oxygen-delivering capacity is directed towards more hypoxic environments (Elmer et al., 2012). Interestingly, the p50 value of M101 is comparable to the p50 value of human hemoglobin HbA inside erythrocytes, and its cooperativity also shows similar properties (Elmer et al., 2012; Batool et al., 2021).

M101 has demonstrated its effectiveness as an additive to organ preservation solutions under both static and perfusion conditions during organ transplantation. Numerous *in vivo* studies

Studies in preclinical models, including various organs such as the heart (Mallet et al., 2014), kidney (Jia et al., 2004; Alix et al., 2020), liver (Lemaire et al., 2019), pancreas (Thuillier et al., 2011), and lung (Glorion et al., 2018), have confirmed the ability of M101 to maintain and improve graft viability and function.

The safety and efficacy of M101 were also confirmed in the OXYOP clinical trial (Clinical Trial Registry No.
NCT 02652520), which concerned a kidney transplant procedure. In this study, HEMO2life® – a class III medical device containing M101 (1 g/L) – was added to the preservative solution (Le Meur et al., 2020). Importantly, no immunological, allergic, or prothrombotic effects associated with the use of M101 were observed. Furthermore, the use of M101 reduced the risk of delayed graft function and improved renal function (Le Meur et al., 2020). These results confirm the safety and efficacy of M101 and indicate its potential clinical applications. Additionally, M101 has been proposed as an immediate substitute for

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blood donation for wounded US Army soldiers (Sen Gupta, 2019).

An interesting use case of HEMO2life® was its application to protect a facial graft during total face retransplantation. In January 2018, the transplantation was performed using a graft protected with HEMO2life®. The operation was successful, and 30 months after the procedure, the patient remains in good condition, with no signs of graft rejection (Lantieri et al., 2020). More recently, M101 has also been proposed as a "molecular ventilator," a potential support for COVID-19 patients in the fight against hypoxemia (Lupon et al., 2021).

Research into this hypothesis could be crucial to developing a potential therapeutic strategy to combat the COVID-19 crisis.

Due to its interesting Oÿ transport capacity, the potential of M101 as an oxygen transporter in various hypoxia-related pathologies requires detailed studies.
Furthermore, its potential as a therapeutic oxygen carrier in situations associated with massive hemorrhage, such as accidents, terrorist attacks, or war injuries, should be thoroughly investigated. The potential use of M101 as a temporary or emergency alternative to reduce the adverse side effects of pathologies requiring frequent blood transfusions requires further investigation. Furthermore, given the recently discovered anti-inflammatory and antibacterial properties of this compound, further research into its therapeutic potential in the treatment of inflammatory and infectious diseases is necessary (Batool et al., 2021).

For artificial blood to be safe and effective, appropriate methods to improve its stability and reduce toxicity are crucial. One of the most important approaches is chemical modification of hemoglobin, for example, through PEGylation, which increases its stability and reduces immunogenicity, reducing the risk of adverse reactions. Another solution is the use of nanotechnology to encapsulate hemoglobin in biocompatible carriers, which prevents its toxic oxidation and prolongs its duration in the body (Gomes et al., 2024).

Better buffering systems play a crucial role in pH regulation and the elimination of free radicals that can lead to cytotoxicity. This makes it possible reducing the negative impact of artificial blood on the body. Furthermore, optimizing the composition of perfluorocarbons allows for improved oxygen solubility while minimizing the risk of adverse effects. Together, these methods contribute to increasing the effectiveness and safety of artificial blood, bringing its practical application in medicine closer (Gomes et al., 2024).

Potential uses of blood substitutes. Research on blood substitutes suggests they could be used in many areas of medicine and science, including:

ÿtreatment of brain injuries and myocardial ischemia,

ÿimproving tissue oxygenation in chronic arterial insufficiency,

ÿextracorporeal perfusion of organs intended for transplantation,

ÿincreasing the effectiveness of cancer radiotherapy by improving tumor oxygenation (Dong and Stowell, 2002).

The production of artificial blood is currently a costly process, which is one of the biggest challenges in its commercialization.
The costs of culturing red blood cells from stem cells are high due to the need for specialized culture media and the lengthy cell multiplication process. Furthermore, technologies based on synthetic oxygen carriers require costly research on their stability and safety.

Currently, producing a unit of artificial blood significantly exceeds the costs of standard transfusions. However, technological advancements and increased production scale may reduce these costs in the future. Further investment in innovative manufacturing methods and optimized production processes is also needed. In the long term, artificial blood could become a cost-effective solution, especially given the growing demand for safe alternatives to traditional transfusions.

Development prospects. Despite previous setbacks, research on artificial blood continues. The future of this technology may include not only the development of effective and safe blood substitutes, but also the development of innovative solutions such as artificial platelets, white blood cells, and red blood cells produced from stem cells. These innovations could constitute

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a response to current problems related to toxicity and limited effectiveness of existing preparations (Kresie, 2001; Sarkar, 2008; Gomes et al., 2024).

The development of artificial blood technology requires Intensive research is underway to increase the efficiency of synthetic oxygen carriers, optimize biotechnological erythrocyte production, and refine biomimetic solutions based on nanotechnology. Progress in these areas may contribute to the creation of alternative blood sources with high clinical efficacy and broad medical applications (Gomes et al., 2024).

Key technologies requiring development include synthetic oxygen carriers, biotechnological production of red blood cells, and the use of nanotechnology and biomaterials. Synthetic oxygen carriers, such as perfluorocarbons and recombinant hemoglobin, represent one of the most promising methods for replacing natural red blood cells. However, their limited oxygen-carrying capacity comparable to natural red blood cells and their short circulation time in the body pose significant technological barriers.

Further refinement of these compounds is necessary to increase their effectiveness and reduce side effects (Gomes et al., 2024).

Biotechnological production of erythrocytes from stem cells is another innovative strategy, but its implementation on a clinical scale faces serious difficulties.
The main challenges include low blood cell production efficiency and high culture costs. Optimization of cell propagation processes, production automation, and the development of more efficient bioreactors can significantly improve the cost-effectiveness of this technology (Gomes et al., 2024).

Nanotechnology and biomaterials represent a promising approach to developing biomimetic erythrocytes that could mimic the natural functions of red blood cells. However, their stability and compatibility with the immune system remain key challenges that require further research. In particular, innovative protective coatings must be developed and their structure optimized to ensure appropriate functionality and safety (Gomes et al., 2024).

Challenges and limitations in the development of red blood cell substitutes. All Red blood cell substitutes that have been clinically tested to date contain components that are not cellular structures. The basic mechanisms of oxygen transport differ from those occurring under physiological conditions. These substitutes disappear from the circulatory system after several hours, or at best, several days, which significantly limits their practical application. Comprehensive data on their safety are lacking (Kim and Greenburg, 2004; Cabrales and Intaglietta, 2013). Clinical trials require extreme caution. Assessing the effectiveness of these preparations is difficult due to the lack of universally accepted evaluation criteria. None of the currently tested preparations has been approved for routine use in humans in Poland, Western Europe, or the United States (Cabrales and Intaglietta, 2013).

Hemopure, developed by Biopure Corp., contains chemically stabilized, cross-linked bovine (cow) hemoglobin in saline solution and is intended for use in humans. The company also developed a similar product under the trade name Oxyglobin for veterinary use in dogs. Oxyglobin is approved in the United States and Europe and was introduced to veterinary clinics and hospitals in March 1998.

Hemopure has been approved in South Africa and Russia (Sprung et al., 2002; Jahr et al., 2021; Chen et al., 2023). It is worth noting that other clinically approved products previously existed, but their sales were discontinued due to side effects (Chen et al., 2023).

Among hemoglobin-based oxygen delivery products, HemAssist, PolyHeme, Hemolink, Hemospan, and Hemoximer have been discontinued. Hemopure is approved for clinical use in Russia and South Africa, and its sister product, Oxyglobin (same formulation as Hemopure but under a different brand name), is approved for veterinary use.

in the United States and the European Union (Jahr et al., 2021). HemO2life® recently received approval for use in organ preservation during transplantation in the European Union, and its developers are seeking approval for the treatment of severe anemia. The OxyVita and Sanguinate products remain in active clinical trials.

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It is hoped that the next 10–20 years will bring dynamic development in the field of oxygen therapies (Chen et al., 2023).

The development of artificial red blood cells has faced significant challenges, primarily due to adverse effects such as toxicity. Several products have received conditional approval in Russia and Africa, but their use is associated with high risks, with mortality rates reaching approximately 50% (Kim et al., 2008).

Summary

Artificial blood, or red blood cell substitutes, represent a promising alternative to traditional blood transfusions, particularly in situations where access to blood is limited or transfusion is impossible. Despite years of intensive research, the development of artificial blood has encountered numerous challenges related to both production technology and safety and efficacy. Existing red blood cell substitutes that have passed clinical trials are characterized by numerous limitations that still prevent their widespread use in medicine.

Current red blood cell substitutes are based on components without a cellular structure, which is a fundamental difference compared to natural blood cells.
These synthetic substitutes perform oxygen transport functions, but the mechanisms they employ differ significantly from the physiological processes occurring in human bodies. Their efficiency in oxygen transport is lower, and their duration in the circulatory system is also short.
Most substitutes disappear from the body within a few hours, and in the best case scenario – after a few days, which significantly limits their practical use in medicine.

The lack of comprehensive data on the safety of blood substitutes is another serious problem. Although some preparations have been approved for use in selected countries, such as South Africa or Russia, their long-term safety, and impact on the body remain incompletely understood. This requires further research, which must be conducted with extreme caution to avoid exposing patients to serious side effects.
Assessing the effectiveness of artificial blood is also difficult due to the lack of universally recognized criteria for evaluating such products. To date, no clear standards have been developed that would allow for the comparison of the effectiveness of various red blood cell substitutes.

Despite these difficulties, some preparations, such as Hemopure, developed by Biopure Corp., have achieved some success. Hemopure, which contains cross-linked bovine hemoglobin in a saline solution, has been approved for use in South Africa and Russia, although its use still carries risks.
Additionally, Oxyglobin, intended for veterinary use, has been approved in the United States and Europe for use in dogs.

It's also important to note that other artificial blood products have received approval in the past, but their sale was halted due to adverse effects, such as toxicity. To date, no product has been approved for widespread use in humans by appropriate authorities, such as the FDA.

The current state of red blood cell substitute technology indicates the need for further development and the search for new, more stable and safe products.
There are many potential avenues for improving the quality of artificial blood. However, challenges related to toxicity, storage life, and production costs remain difficult to overcome. Therefore, while artificial blood offers enormous potential in medicine, its full widespread use may require many years of research and further technological development.

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ÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ: ÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿ, ÿÿ potency key uses and maintenance tips. The problem is the lack of donor funds in banks and their growth. ÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ. ÿ ÿÿ'ÿÿÿÿ ÿ ÿÿÿ ÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ effective lovers of cows, how they could manage their functions ÿÿÿÿÿÿÿÿÿ information about the organization and the changes in the traditions donors. ÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿ, ÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿ, ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿ nanotechnology. ÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿ synthetic erythrocytes.

ÿÿÿÿÿÿÿÿÿÿ. ÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿ comprehensive information ÿÿÿÿÿÿÿÿÿ ÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ, ÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿ ÿÿÿÿÿ, ÿÿÿÿ ÿÿ PubMed, Scopus, Web of Science ÿÿ Google Scholar. I have read more about scientific research, I have read about it ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿ, ÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿ new ÿÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ. ÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ erythrocites, ÿÿÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿÿÿ, ÿÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿ (napriclade, recombinant geoglobe, non-recombinant), synthetic microchastins, ÿÿ ÿÿÿÿÿÿÿÿ eritrocytes, ÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿÿÿÿ. ÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ, how to learn information about security, effectiveness and possible ÿÿÿÿÿÿÿÿ ÿÿÿ ÿÿÿÿÿÿÿÿÿ.

Scientific novelty. ÿÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿ ÿÿÿÿÿÿ ÿÿÿÿ, ÿÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿ, ÿÿÿ'ÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿÿ. ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ, ÿÿÿÿ ÿÿ effective locks ÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿ'ÿ, ÿÿ bodybuilding, ÿÿÿÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ effects. ÿÿÿÿ ÿÿÿÿ, ÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿ to take care of such medical products for patients. ÿ ÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ and technological developments, how they can be implemented ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿ. ÿÿÿÿÿÿÿÿÿÿ brain, ÿÿÿÿÿÿÿ used nanotechnology, biotechnology and materials ÿÿÿ created and safe cows.

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ÿÿÿÿÿÿÿÿ. ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿ new media, ÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ refining ÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿ (ÿÿÿÿÿÿÿÿÿ, ÿ ÿÿÿÿÿÿÿ agreements, ÿÿÿ ÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿ, ÿ ÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ to donation cows). Not to mention the technical aspects of the baggage ÿÿÿÿÿÿÿ, bagato ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿ, ÿÿÿ'ÿÿÿÿÿÿÿ ÿ no longer stable, no toxic effects efficient transportation of pickers. ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ, ÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿ, Demonstration of perspective properties, ÿÿÿÿÿ ÿÿÿÿ ÿÿÿ ÿÿ from far away ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ. ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿ ÿÿ, ÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿ ÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿ on ÿÿÿÿÿÿÿÿÿ bezpeki, ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ on viral diseases. ÿÿÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿ ÿÿÿÿÿ ÿ ÿÿÿÿÿÿÿÿÿÿÿÿÿÿÿ, ÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ, ÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿ, ÿ ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿ ÿ ÿÿÿÿÿÿ ÿÿÿÿÿ.

ÿÿÿÿÿÿÿ ÿÿÿÿÿ: ÿÿÿÿÿÿ ÿÿÿÿ, ÿÿÿÿÿÿÿÿÿÿÿÿÿÿ, ÿÿÿÿÿÿÿÿ used, ÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿ, ÿÿÿÿÿÿÿ ÿÿÿÿÿÿÿÿÿÿÿÿ ÿÿÿÿÿÿ, ÿÿÿÿÿÿÿÿÿÿÿÿÿÿ, ÿÿÿÿÿ ÿÿÿÿÿ ÿÿ ÿÿÿÿÿÿ geoglobe

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